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Research Article

Antioxidant Acitivity *in vitro* and *in vivo* of Polysaccharide Isolated from Pumpkin

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Abstract: A water-soluble polysaccharide named as PP-e was obtained from pumpkin by the water and ethanol extract, deproteinization, alcohol grading, gel chromatography. The average molecular weight of PP-e was 4.63 kDa determined by HPGPC. PP-e was a heteropolysaccharide by the analysis of GC and consisted of L-rhamnose L-arabinose, D-glucose and D-galactose in a molar ratio of 1.12:5.19:1.00:3.91. The antioxidant activity of PP-e *in vitro* and *in vivo* was also evaluated. *In vitro* antioxidant assay, PP-e showed strong inhibition of superoxide radical, hydroxyl radical and DPPH radical. For antioxidant testing *in vivo*, PP-e was administrated by intraperitoneal injection with the dosage of 150 mg/kg in alloxan-induced mice model. As results, PP-e could significantly inhibit the formation of malondialdehyde and nitric oxide in mice livers and raised the activities of antioxidant enzymes in mice livers and serums. The results suggest that the polysaccharide from pumpkin has direct and potent antioxidant activities.

Keywords: Alloxan, antioxidant activity, monosaccharide composition, polysaccharide, pumpkin, purification

INTRODUCTION

Free radicals can usually be generated by several biological reactions in the body and capable of damaging crucial bio-molecules; if they are not scavenged effectively by cellular constituents, they will lead to disease conditions (Aruoma, 1994; Benzie, 2000; Halliwell *et al*., 1992). Recently, there is more focus on finding the effective and natural antioxidants to reduce these damages to our bodies since the synthetic antioxidants were used (Zhang *et al*., 2011). Current researches into free radicals have confirmed that foods rich in antioxidants play an essential role in the prevention of cardiovascular diseases and cancers and neurodegenerative diseases, as well as inflammation and problems caused by cell and cutaneous aging (Benzie, 2000; Finkel and Holbrook, 2000). Natural antioxidants may inhibit lipid peroxidation in food and improve food quality and safety. Several plants extracts have been shown to have antioxidant activity (Amarowicz *et al*., 2004; Duan *et al*., 2006). Polysaccharides, distributed widely in animals, plants and microorganisms, have been demonstrated to play an important role as dietary free radical scavenger in the prevention of oxidative damage in living organism (Tsiapali *et al*., 2001; Pang *et al*., 2000).

The pulp of pumpkin, is a plant that has been used frequently as functional food or medicine. Pumpkin belongs to the family Cucubitaceae and consists of succulent stem with numerous seeds (Saganuwan, 2009). Preliminary investigations have proven that a pumpkin-rich diet has many pharmacological activity (Zhang *et al*., 2002; Zhang and Yao, 2002; Adamsa *et al*., 2011). Zhu *et al*. (2007) demonstrated that pumpkin polysaccharides could cut down the blood glucose of the diabetic rats, enhance the SOD activity and improve the ultrastructural features of islet cell. However, there is not any systematic investigation about the antioxidant activity of the polysaccharides from pumpkin both *in vitro* and *in vivo*. Therefore, evaluation of the antioxidant activity of the polysaccharide from pumpkin will be important for the elucidation of function and utilization of the polysaccharide.

Alterations in the antioxidant enzyme activities and increased oxidative damage have been demonstrated in different tissues of diabetic animals (Morales and Jiménez Pérez, 2001). To our knowledge, there is no available data on livers and serums Glutathione Reductase (GR), catalase (CAT), Superoxide Dismutase (SOD) activities and Nitric Oxide (NO), Malondialdehyde (MDA) concentrations in mice with diabetes mellitus. In the present study, we measured the activities of SOD, CAT and GR in serums and livers and MDA and NO concentrations in livers of alloxan-induced model mice. The aim of present study is to evaluate the antioxidant activities of the polysaccharide from pumpkin, we report in detail the antioxidant activities of the polysaccharide from pumpkin by using alloxan-induced model mice as *in vivo* model and different extracorporeal antioxidant methods.

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MATERIALS AND METHODS

Plant materials and chemicals: Alloxan was purchased from Sigma Chemical Co.; Sephacryl s-200 was purchased from GE Ltd. US; Standard sample of molecular weight of Dextran was purchased from SHODEX Ltd. Japan; 1,1-Diphenyl-2-picryldydrazyl (DPPH) was purchased from Sigma Chemical Co.; Assay kits for malondialdehyde (MDA), catalase (CAT), Superoxide Dismutase (SOD), Glutathione Reductase (GR) and Nitric Oxide (NO) were the products of Nanjing Jiancheng Institute (Nanjing, China); All other chemicals used were of analytical grade.

Pumpkins were purchased from the local market (Hangzhou, China). The pumpkins we used in this experiment were cultivated in a suburban district of Hangzhou, China. Its botanical name is *Cucurbita moschata*. This species of pumpkin is unique and very common in the southeast part of China. Uniformity of shape and maturity fresh fruit bodies of pumpkin was selected. The fresh pumpkin was peeled, seeded and sliced into pieces (0.5 cm*2.0 cm*5.0 cm). The slices were dried under sun light for about two days until completely dry. Dried slices were ground into powder.

Animals: Young adult (6-7 weeks) Kunming mice of single sex, weighing 20-25 g, were obtained from the Zhejiang Academy of Medical Science (Hangzhou, China) and adopted for 7 days to controlled experimental conditions: temperature 25±2°C, humidity 55-60% and a 12-h light/dark cycle. They were kept in cages (six animals per cage) and randomly distributed in each experimental group. Access to food and tap water was freely allowed. The approval of this experiment was obtained from the Institutional Animal Ethics Committee of China Jiliang University (Hangzhou, China).

Preparation of polysaccharides samples: Pumpkin powder was mixed with distilled water at a ratio of 1: 30 (w/w) at 60°C for 2 h before centrifugation at 3000 rpm for 20 min. The supernatant was collected as water soluble extract while the sediment was mixed with 95% alcohol at a ratio of 1:3 (w/w) for 48 h at 4°C and followed by centrifugation at 3000 rpm for 10 min. The sediment was discarded while the supernatant, the alcohol soluble extract, was mixed with the previously collected water soluble extract and purified by chloroform and n-butyl alcohol step by step. Deproteinization was performed with sevag' reagent. 95% Ethanol was continually added slowly to final concentration of 30%, the collected precipitate was termed PP-a (2.17%); Similarly, the precipitates PP-b (3.24%), PP-c (50.90%) and PP-d (17.43%) were obtained by adding Ethanol to concentration of 50, 70 and 90%, respectively successively. Finally, four fractions of crude polysaccharides were obtained by lyophylization. PP-c was purifyby by gel chromatogram obtained two components of different molecular weight were termed PP-e (15.34%) and PP-f (20.76%).

Analysis of PP-e monosaccharide:

Condition of monosaccharide hydrolysis: Weighed 20 mg of pumpkin polysaccharide powder into ampoule bottle, added 2 mL 2 mol/L HCL and seal the bottle. Put the bottle under 105°C for 4 h, adding 200 g/L NaOH to neutralize after cooling. Used distilled water to dialyze for 24 h and then vacuum drying.

Condition of monosaccharide derivatization: Took some dried sample into 2 mL Eppendorf (EP) tube, adding 0.6 mL pyridine and 100 mg hydroxylamine hydrochloride. Then put it in 90°C thermostatic water for 30 min; cooled down and added 0.6 mL acetic oxide, put it in 90°C thermostatic water for another 30 min. After cooling down, used membrane to filter the supernatant fluid and got prepared for using.

Condition of gas chromatography: OV-1701 quartz capillary column (30 m×Φ0.32 mm) was made by Shimadzu Ltd. Japan; FID detector; temperature programming: 150°C(7°C/min)→190°C(15°C/min)→ 250°C(7min); temperature of injection: 280°C; temperature of detector 260°C; flow rate: 1.5 mL/min; injection volume: 1 μL.

Preparation for standard monosaccharide solution: Weighed glucuronic acid, fucose, xylose, mannose, glucose, galactose, glucosamine 250.00 mg into 25 mL volumetric flasks, adding anhydrous pyridine to scale mark. Derived them according to the method above. Mixed the derived solution and used pyridine to dilute into 6 different concentration. Filtered through membrane and prepared them for using.

Determination of PP-e molecular weight: Diluted the solution to 10 mg/mL and injected it. Used TSK gel super multi PW-M column, RI detector, pure water as mobile phase; column temperature was 40°C; flow rate was 0.6 mL/min; injection volume was 20 μL and recorded the chromatograph curve. Injected standard dextran of 6 different molecular weight Mw = 158100, Mw = 91100, Mw = 31200, Mw = 20100, Mw = 4300, Mw = 1200, Mw = 505, Mw = 180 based on the same HPLC condition. Recorded every V_R and drew the standard curve based on V_R as the X-axis and LogM as the Y-axis. Injected the sample according to the condition mentioned above and got V_R and calculated the molecular weight of polysaccharide according to the equation of linear regression of standard curve.

DPPH· free radical-scavenging activity test: Radical-scavenging activity against the stable radical DPPH was determined following the scientific literature (Kakkar *et al*., 1995). In brief, the solution of DPPH· in ethanol

(0.2 mM) was prepared daily, before UV measurements. An aliquot of sample (0.25-2.0 mg/mL, 2 mL) was thoroughly mixed with 2 mL of freshly prepared DPPH· and kept in the dark for 30 min at room temperature (25°C) and then the absorbance was measured (U-2000, Hitachi, Japan) at 517 nm. The experiment was carried out in triplicate. The ability to scavenge the DPPH· radical was calculated by the following formula:

$$\text{Scavenging effect (\%)} = [1-(A_s-A_0/A_1)] \times 100$$

where, the A_1 is the absorbance of the control (DPPH· solution without sample), the A_s is the test sample (DPPH· solution plus test sample) and the A_0 is the absorbance of the sample only (sample without DPPH· solution).

Hydroxyl radical-scavenging activity test: The hydroxyl radical-scavenging assay was carried out following the scientific literature (De Avellar *et al*., 2004) with some modifications. Both sodium salicylate (9 mM, 0.4 mL) and $FeSO_4$ (9 mM, 2 mL) were dissolved in phosphate buffer (pH 7.4) and mixed thoroughly. H_2O_2 (0.1%, 1.0 mL) and samples (0.1-0.4 mg/mL, 0.4 mL) were added. The mixture was incubated at 37°C for 60 min and the absorbance was measured at 510 nm. Results were determined using the following equation:

$$\text{Scavenging activity (\%)} = [(A_S-A_1)/(A_0-A_1)] \times 100$$

where, the A_S is the absorbance of the sample, the A_1 is the absorbance of control solution containing sodium salicylate, $FeSO_4$ and H_2O_2 and the A_0 is the absorbance of blank solution containing sodium salicylate and $FeSO_4$.

Superoxide radical-scavenging activity test: The scavenging effect on superoxide radical was assayed by using the scientific literature (Stewar and Beewley, 1980) with some modifications. The 0.25 mL test sample (2 mg/mL) was added to the reaction mixture to give a final volume of 5.0 mL Tris-HCl buffer (pH 8.0), contained the reagent at final concentration: 10 mM pyrogallol. The mixture was incubated at 25°C for 6 min and then the absorbance was measured at 325 nm. The effect of scavenging the superoxide radical was calculated using the following equation:

$$\text{Scavenging effect (\%)} = (1-A_s/A_1) \times 100$$

where, the A_S is the absorbance of the sample, the A_1 is the absorbance of control solution containing each reagent.

Determination of antioxidant activity of PP-e *in vivo*:

Animal selection and experimental design: A total of 18 mice were equally divided into three groups randomly including Normal Control Group (NCG), alloxan model control group (ACG) and PP-e group. Mice in NCG were given 15 mL/kg (body weight) physiological saline solution (0.9% w/v) per day through intraperitoneal injection. Mice in ACG and PP-e were fasted overnight and induced by a rapid intravenous injection of alloxan (200 mg/kg) freshly dissolved in sodium chloride injection. Then the mice were given 5% glucose in drinking water overnight to prevent hypoglycemia. Alloxan-induced mice were allowed free access to food and water until starting the experiment. Seventy-two hours later, only mice with blood glucose levels higher than 11.3 mmol/L were selected as the alloxan-induced mice (Serradas *et al*., 1998) and blood glucose levels were measured by the one touch ^{TM} II. The mice in ACG and PP-e group were given physiological saline solution and PP-e by a rapid intravenous injection, respectively.

Assay of SOD, GSH-Px, MDA and CAT: After overnight fasting following the drug administration, the mice were sacrificed by decapitation. Blood samples were centrifuged for 10 min (4000 g at 4°C) to afford the serums required. The activities of SOD, CAT and GR in serum were measured according to the instructions on the kits.

The liver was excised, weighed and homogenized in 0.1 g/mL of ice-cold isotonic physiological saline based on wet weight. The suspension was centrifuged for 10 min (4000 g at 4°C); the supernatant was subjected to the measurement of the activities of SOD, CAT, GR and NO and the levels of MDA.

RESULTS

Analysis of molecular composition of PP-e: Table 1 shows reserve time of standard monosaccharide and Fig. 1 shows GC chromatogram of PP-e.

Based on the mixed monosaccharide standard equation, PP-e is composed of rhamnose, arabinose, glucose and galactose. The approximate mole ratio of rhamnose: arabinose: glucose: galactose = 1.12:5.19: 1.00: 3.91.

Table 1: Mixed monosaccharide standard equation of regression

Monosaccharides	Reserve time/min	Equation of regression	R^2	Linear range
Rhamnose	10.132	Y = 56137x+3566.3	0.9984	0.5-109.8 μmol/mL
Fucose	10.643	Y = 90828x-8265.5	0.9954	0.3-133.2 μmol/mL
Arabinose	10.803	Y = 46246x+2011.1	0.9983	0.3-52.2 μmol/mL
Mannose	12.935	Y = 89954x-6919.3	0.9986	0.2-47.6 μmol/mL
Glucose	13.313	Y = 85083x-8765.1	0.9985	0.2-43.3 μmol/mL
Galactose	13.520	Y = 79545x-8697.5	0.9976	0.2-47.6 μmol/mL

Y: refers to peak area; x: the concentration of monosaccharide mmol/mL

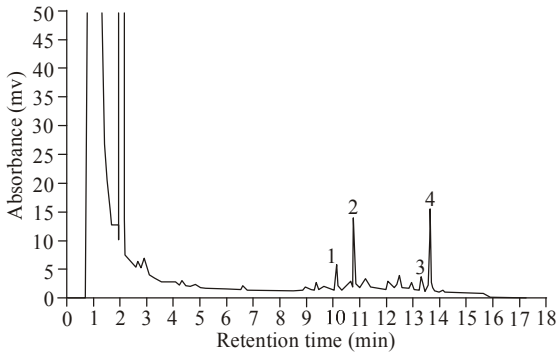

Fig. 1: GC chromatogram of PP-e

Molecular weight of PP-e:

Standard curves of molecular weight of series of dextran: Figure 2a shows the equations and curves of standard dextran: Fitted equation based on the relationship between molecular weight of standard dextran and reserved volume: V_R = -1.1442 LogM+12.65 (R^2 = 0.996).

HPLC chromatogram of PP-e (Fig. 2b): According to the fitted equation based on the relationship between molecular weight of standard dextran and reserved volume: V_R =-1.1442 *Log*M+12.65 (R^2 = 0.996), it can be calculated that the molecular weight of PP-e is 4.63 kDa. For the pure polysaccharide sample, its microstructure is not uniform but only represented average distribution of similar chain length of polysaccharides. Therefore, pure polysaccharide sample actually means the uniform composition of polysaccharide in a certain range of relative molecular mass. Experiment showed that PP-e was widely distributed in the HPLC chromatogram also explains this phenomenon.

Antioxidant activities of PP-e *in vitro*:

DPPH· scavenging activity: DPPH is a useful reagent for investigating the free radical-scavenging activities of materials. In the DPPH· test, the antioxidants were able to reduce the stable DPPH to the yellow-coloured dipheny lpricry lhydrazine. The DPPH· free radical-scavenging activities of PP-e with different concentration were shown in Fig. 3. As can be seen in Fig. 3a, the PP-e exhibited a rather strong concentration-dependent DPPH· radical-scavenging activity. It was noted that, when the concentration of PP-e were increased from 0.25 to 2.0 mg/mL, the antioxidant activity increased. At concentration of 1 mg/mL, scavenging effect of PP-e was 26.05%.

Košťálová *et al*. (2010) indicated that the overall antioxidant activity in the 2, 2-diphenyl-1-picrylhydrazyl (DPPH·) assay of fractions from pumpkin (*Cucurbita pepo* L. var. *Styriaca*) extraction by the classical and ultrasound-assisted extractions was very low. In contrast, the pumpkin pectin/hemicellulose fractions, exhibited considerable uEfficient Concentrations"ffic$_{50}$) ranged from 14 to 21 when compared to that of quercetin (EC_{50} = 0.14) used as positive control. EC_{50} is defined as the concentration of substrate that causes 50% loss of the DPPH activity. The results suggested that the pumpkin polysaccharide fractions represented dietary fibers with a high content of pectin and/or beneficial antioxidant properties, which might be suitable for functional food applications:

Hydroxyl radical assay: Hydroxyl radical is considered to be a high potent oxidant, which can react with all biomacromolecules functioning in living cells (Gülçin *et al*., 2010). As shown in Fig. 3b, PP-e had the abilities to scavenge hydroxyl radicals at concentrations from 0.4 to 4 mg/mL. The scavenging abilities increased with the concentration increasing. The scavenging effect of PP-e was 95.35% at the concentration of 0.4 mg/mL. It has been reported that scavenging effect on hydroxyl radical of Vitamin C was less than 20% at 0.63 mg, namely 6.3 mg/mL (Luo and Fang, 2008). Therefore, the polysaccharides from pumpkin had significant effect on scavenging hydroxyl radical.

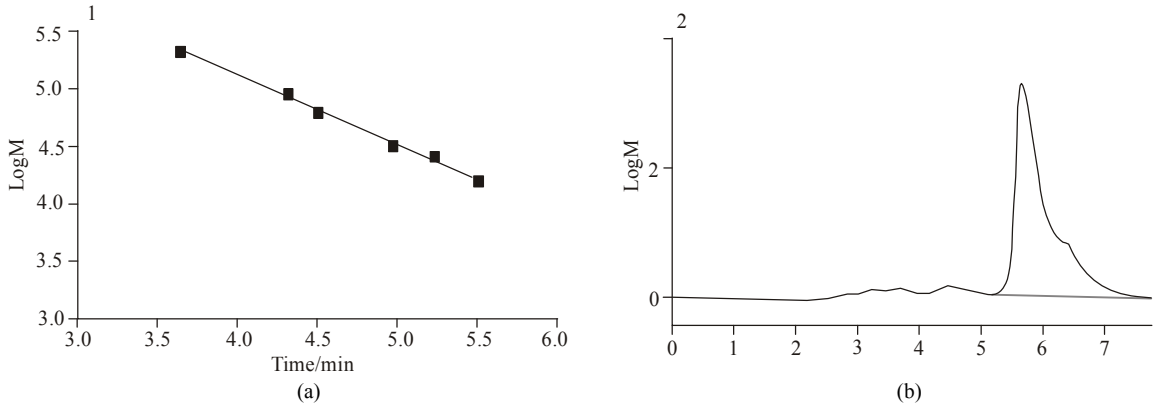

Fig. 2: Standard curve and PP-e of determination of molecular weight

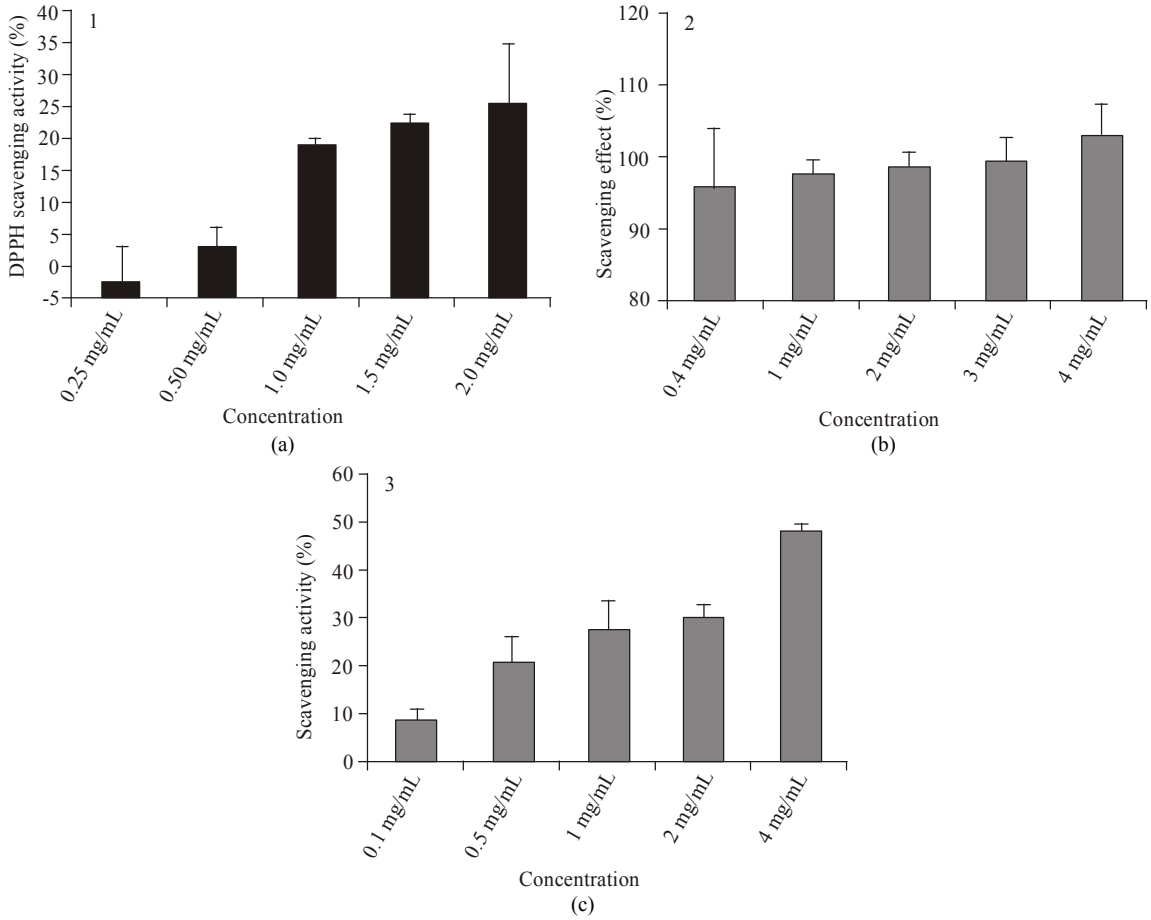

Fig. 3: Antioxidant ability of PP-e

Superoxide radical assay: Superoxide radical is one of harmful species to cellular component as a precursor of more reactive oxygen species. The superoxide radical is known to be produced *in vivo* and can result in the formation of H_2O_2 via dismutation reaction. Moreover, the conversion of superoxide and H_2O_2 into more reactive species, e.g., the hydroxyl radical, has been thought to be one of the unfavourable effects caused by superoxide radicals (Halliwell, 1978). In the present experiment, the PP-e was effective in superoxide radical generated *in vitro* scavenging assay also in a concentration-dependent manner (Fig. 3c). At concentration of 4 mg/mL, scavenging effect of PP-e was 50.52%. This ability of polysaccharides from pumpkin to scavenge superoxide may contribute to its significant antioxidant potential. The increase in superoxide anion radical-scavenging activity of polysaccharides of different concentration is due to increase in number of hydroxyl groups in the molecule.

The antioxidant activity of pumpkin polysaccharides *in vivo*:

Effect of PP-e on the activities of antioxidant enzymes in liver: As shown in Table 2, a marked increase in MDA and NO levels ($p<0.01$) and decrease of antioxidant enzymes activities (SOD, GR, CAT) ($p<0.05$) were observed in liver between the treatments of NCG and ACG. PP-e treatment inhibited

Table 2: Effects of PP-e on the activities of SOD (U/mg protein), CAT (U/mg protein), GR (U/g protein), NO (μmol/g protein) and levels of MDA (nmol/mg protein) in livers[a]

	Group I (NCG)	Group II (ACG)	Group III (PP-e)
SOD	204.02 ± 2.34^{Bb}	103.79 ± 7.08^{Aa}	204.61 ± 5.50^{Bb}
CAT	31.99 ± 1.43^{Bb}	18.64 ± 1.41^{Aa}	32.70 ± 2.16^{Bb}
GR	25.06 ± 11.46^{Bb}	8.56 ± 1.85^{Aa}	19.72 ± 4.87^{Bb}
NO	170.10 ± 19.18^{Ba}	356.22 ± 3.67^{Cb}	147.99 ± 5.19^{Aa}
MDA	3.61 ± 1.43^{Aa}	12.69 ± 2.64^{Bb}	6.15 ± 0.74^{A}

[a]: Data were presented as means±SD ($n = 6$) and evaluated by one-way ANOVA followed by the Student-Newman-Keuls test to detect inter-group differences; Differences were considered to be statistically significant if $p<0.05$; A, B, C, $p<0.05$, compared with model group; a, b, $p<0.01$; compared with model group

Table 3: Effect of PP-e on the activity of SOD (U/mg protein), CAT (U/mg protein), GR (U/g protein) in serums[a]

	Group I (NCG)	Group II (ACG)	Group III (PP-e)
SOD	150.52 ± 20.52^{Ba}	106.22 ± 26.44^{Aa}	151.64 ± 15.06^{Ba}
CAT	16.08 ± 5.06^{Bab}	5.47 ± 3.59^{Aa}	19.75 ± 6.26^{Bb}
GR	65.50 ± 10.04^{Aa}	41.00 ± 10.05^{Aa}	55.95 ± 20.22^{Aa}

[a]: Data were presented as means±SD ($n = 6$) and evaluated by one-way ANOVA followed by the Student-Newman-Keuls test to detect inter-group differences; Differences were considered to be statistically significant if $p<0.05$; A, B, C, $p<0.05$; compared with model group; a, b, $p<0.01$; compared with model group

significantly the formation of MDA and NO in mice livers and raised the activities of antioxidant enzymes (SOD, GR, CAT) ($p<0.05$).

Effect of PP-e on the activities of antioxidant enzymes in serums: As shown in Table 3, administration of PP-e elevated the activities of antioxidant enzymes (SOD, GR, CAT) ($p<0.05$) in serums and a marked decrease of antioxidant enzymes activities (SOD, GR, CAT) ($p<0.05$) were observed in serums between the treatments of NCG and ACG.

DISCUSSION

Cucurbita moschata, comprised of a large amount of polysaccharides, exhibits potent antioxidant and antidiabetic properties. Several studies have demonstrated the involvement of free radicals in the genesis of diabetes mellitus and their role in the induction of lipid peroxidation and depression of antioxidant defense system (Venkateswaran and Pari, 2002; Ananthan *et al*., 2004). In the present study, the antioxidant potential of PP-e in alloxan-induced diabetic mice was examined. Alloxan is commonly used to produce diabetes mellitus in experimental animals because of its ability to destroy the ß-cells of pancreas possibly by an excessive generation of reactive oxygen species such as, H_2O_2, O_2^{-} and $HO^{\cdot}$ (Dunn *et al*., 1943). Free-radical-induced lipid peroxidation has been associated with a number of diseases, including diabetes mellitus (Feillet-Coudray *et al*., 1999). Selective damage of islet cells in Type I diabetes could probably be due to low levels of antioxidant enzymes (superoxide dismutase, catalase and glutathione peroxidase) in the pancreas (Kakkar *et al*., 1998). The major antioxidant enzymes, including Superoxide dismutases (SOD), Glutathione Reductase (GR) and Catalase (CAT), are regarded as the first line of the antioxidant defense system against ROS generated *in vivo* during oxidative stress. SOD reduces superoxide levels in the cell; these enzymes catalyze the conversion of the superoxide radical to molecular oxygen and hydrogen peroxide. CAT is ubiquitously expressed amongmammalian tissues and is primarily located in the peroxisomes. The primary catalytic function of catalase is the decomposition of hydrogen peroxide to oxygen and water (Halliwell and Gutteridge, 1989). In the present study, we found that SOD, GR and CAT activities decreased markedly in group ACG and these changes had statistical significance in livers and serums. It is likely that the decrease in the activities of SOD and GR is the main factor in lipid peroxidative damage.

The pumpkin polysaccharide showed inhibiting ability for lipid peroxidation as observed in the reduction of MDA production. The result was consistent with that of pumpkin polysaccharide *in vitro*. The administration of pumpkin polysaccharide induced the activities of SOD, GR and CAT. The enhanced activities of SOD, GR and CAT could be very effective in scavenging various types of oxygen free radicals and their products. So the inhibitory effect of the pumpkin polysaccharide on lipid peroxidation might be attributed to their effects on the antioxidant enzymes and non-enzymatic system.

Over the past decade or so, it has become evident that the free radical gas Nitric Oxide (NO) acts as a novel transcellular messenger molecule in many key physiological and pathological processes (Xu *et al*., 2002). Endogenous NO is of a dual role in specialized tissues and cells, which is an essential physiological signaling molecule not only mediating various cell functions but also induces cytotoxic and mutagenic effects when present in excess. NO reacts rapidly with superoxide anion to form peroxynitrite, which may be cytotoxic by itself or easily decompose to the highly reactive and toxic hydroxyl radical and Nitrogen Dioxide (NO_2). The pumpkin polysaccharide showed inhibiting ability for present in excess as observed in the reduction of NO production.

CONCLUSION

This study investigated the activities of GR, SOD and CAT and the concentrations of NO and MDA in livers with alloxan-induced diabetic mice, also the activities of GR, SOD and CAT in serums were investigated. The results showed that lower SOD, CAT and GR activities in serums and livers and higher NO and MDA concentrations in livers of alloxan-induced diabetic mice compared to the control group. The administration of pumpkin polysaccharide induced the activities of SOD, GR and CAT in serums and livers and reduced the production of MDA and NO for present in excess. Our present study reveals that the pumpkin polysaccharide had the antioxidative potential in alloxan-induced diabetic mice. The evidence suggests that this polysaccharide could be beneficial for protection against diabetes and its oxidative stress. Further studies need to be carried out in order to define more accurately and more precisely the active ingredient.

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